## **FIGURES**

```
MKMNVESFNLDHTKVKAPYVRIADRKKGVNGDLIVKYDVRFKOPNRDHM-DMPSLHSLEHLVAEIIRNHAN-----YVVDWSPMGC
D. radiodurans MPDMANVESFDLDHTKVKAPYVRLAGVKTTPKGDQISKYDLRFLQPNQGAI-DPAAIHTLEHLLAGYMRDHLE-----GVVDVSPMGC
H. influenzae MPLLDSFKVDHTKNNAPAVRIAKTMLTPKGDNITVFDLRFCIPNKEIL-SPKGIHTLEHLFAGFMRDHLN-GDSIEIIDISPMGC
                    MPLLDSFKVDHTKMPAPAVRLAKVMKTPKGDDISVFDLRFCIPNKDIM-SEKGTHTLEHLFAGFYRDHLN-SNSVEIIDISPMGC
   jejuni
                    MKK_TSFTIDHTKLN-PGIYVS-RKDTFENVIFTTIDIRIKAPNIEPIIENAAIHTIEHIGATLLRNNEV--WTEKIVYFGPMGC
B. burgdorferi
                   MVKVESFELDHTKVKAPYVRKAGIKIGPKGDIVSKFDLRFVQPNKELL-SDKGMHTLEHFLAGFMREKLD-----DVIDISPMGC
MPLLDSFKVDHTRMHAPAVRVAKTMTTPKGDTITVFDLRFCVPNKEIL-PEKGIHTLEHLFAGFMRDHLN-GNGVEIIDISPMGC
C. perfringens
N. meningitidis
                    MPLLDSFAVDHTRMQAPAVRVAKTMNTPHGDAITVFDLRFCIPNKEVM-PEKGIHTLEHLFAGFMRDHLN-GNGVEIIDISPMGC
  tvphimurium
V. harveyı
                    MPLLDSFTVDHTRMNAPAVRVAKTMQTPKGDTITVFDLRFTAPNKDIL-SEKGIHTLEHLYAGFMRNHLN-GDSVEIIDISPMGC
E. coli
                    MPLLDSFTVDHTRMEAPAVRVAKTMNTPHGDAITVFDLRFCVPNKEVM-PERGIHTLEHLFAGFMRNHLN-GNGVEIIDISPMGC
V. cholerae
                    MPLLDSFTVDHTRMNAPAVRVAKTMQTPKGDTITVFDLRTMQPKDILS-ERGAIHTLEHYLAFYMRNHLN-GSQVEIIDISPMGC
                   MPSVESFELDHNAVVAPYVRHCGVHKVGTDGVVNKFDIRFCQPNKQAM-KPDTIHTLEHLLAFTIRSHAEKYDHFDIIDISPMGC
B. subtilis
B. halodurans
                   MPTVESFELDHTIVKAPFVRPCGTHKVGTNGEVNKFDIRFFQPNKQAM-KPDVIHTLEHLLALNIRKFAEAYDHFDVIDLSPMGC
Numbering
                         10
                                   20
                                             30
                                                        40
                                                                   50 -
                                                                              60
                                                                                                     80
Secondary Structure
                                     SSSSSSSS
                                                    SSSSSSS
                                                                        нининининининини
                                                                                                    SSSSSS
                        3333
                                                                                                      S3
                                        S1
                                                       S2
                 QTGFYLTVLNHDNYTE1LEVLEKTMQDVLKAK---EVPASNEKQCGWAANHTLEGAQNLARAFLDKRAEWSEVGV
H. pylori
                RTGMYMAVIGEPDEQGVMKAFEAALKDTAGHD-Q-PIPGVSELECGNYRDHDLAAARQHARDVLDQGLKVQETILLER
D. radiodurans
H. influenzae
                 {\tt RTGFYMSLIGTPNEQKVSEAWLASMQDVLGVQDQASIPELNIYQCGSYTEHSLEDAHEIAKNVIARGIGVNKNEDLSLDNSLLK}
   jejuni
                 RTGFYMSLIGTPDEKSIAKAWEAAMKDVLSVSDQSKIPELNIYQCGTCAMHSLDEAKQIAQKVLNLGISIINNKRLKLENA
                B. burgdorferi
C. perfringens KTGFYLTSFGDIDVKDIIEALEYSLSKVLE---QEEIPAANELQCGSAKLHSLELAKSHAKQVLENGISDKFYVE
N. meningitidis RTGFYMSLIGTPSEQQVADAWLASMQDVLNVKDQSKIPELNEYQCGTYQMHSLAEAQQIAQNVLARKVAVNKNEELTLDEGLLNA
S. typhimurium RTGFYMSLIGTPDEQRVADAWKAAMADVLKVQDQNQIPELNVYQCGTYQMHSLSEAQDIARHILERDVRVNSNKELALPKEKLQELHI
                 RTGFYMSLIGTPSEOOVADAWIAAMEDVLKVENONKIPELNEYOCGTAAMHSLDEAKOIAKNILEVGVAVNKNDELALPESMLRELRID
V. harveyi
                 RTGFYMSLIGTPDEQRVADAWKAAMEDVLKVQDQNQIPELNVYQCGTYQMFSLQEAQDIARSILERDVRINSNEELALPKEKLQELHI
E. coli
V. cholerae
                 RTGFYMSLIGAPTEQQVAQAWLAAMQDVLKVESQEQIPELNEYQCGTAAMFSLEEAKAIAKNVJAAGISVNRNDELALPESMLNELKVH
                 QTGYYLVVSGEPTSAEIVDLLEDTMKE-AVEIT--EIPAANEKQCGQAKLHDLEGAKRLMRFWLSQDKEELIKVFG
B. subtilis
                 QTGFYLIMSGKPTVEEIIDVLEQTMKYSLELE---EVPAANEKQCGQAKLHDLDGAKKLMTYWLSHEKDSLTKVFES
B. halodurans
                                                                      140
                            100
                                                 120
                                                           130
                                                                                           160
                                                                                                      170
                                                                        нининининин
                    SSSSSS
                              нинининининин
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Figure 1 Sequence alignment of LuxS proteins. Color coding: red = greater than 92% identity (or homology in the case of F/Y, S/T, or D/E) and green = hydrophobic residues (A/V/I/L/M/W/Y/F). At the bottom is indicated the residue numbering employed as well as the common secondary structure elements determined in this invention with 3 = 3/10 helix, S = beta strand, and H = alpha helix.

FIG. 2. Representative diffraction image from a *H. pylori* LuxS crystal analyzed in this patent.

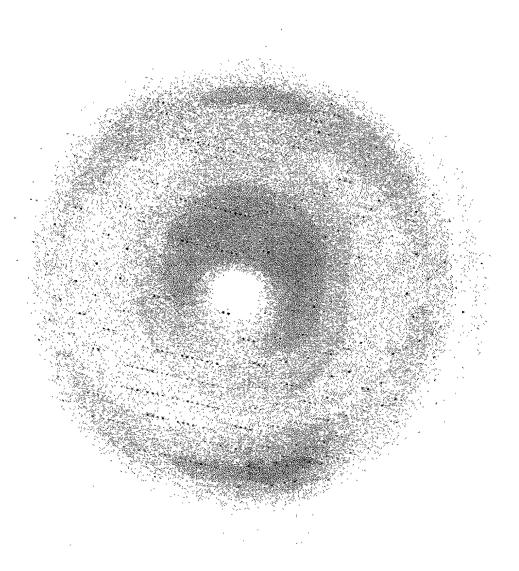


FIG 3. Representative diffraction image from a *H.influenzaei* LuxS crystal analyzed in this patent.

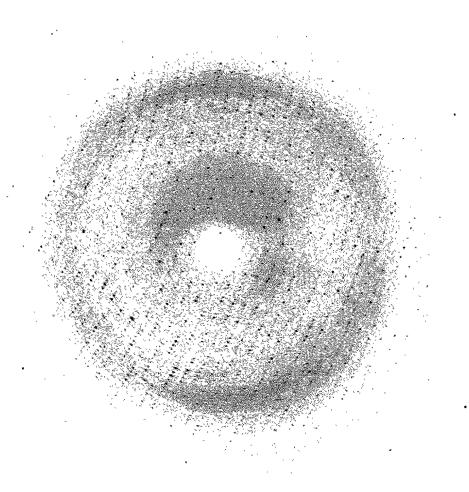


FIG 4. Representative diffraction image from  $P2_1$  spacegroup D. radiodurans LuxS crystals analyzed in this patent.

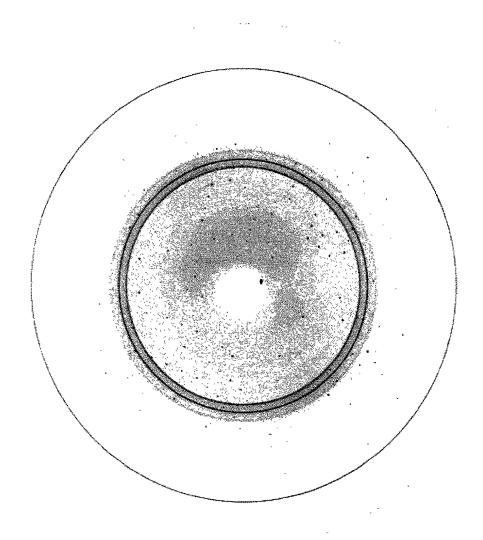


FIG 5. Representative diffraction image from C2 spacegroup D. radiodurans LuxS crystals analyzed in this patent.

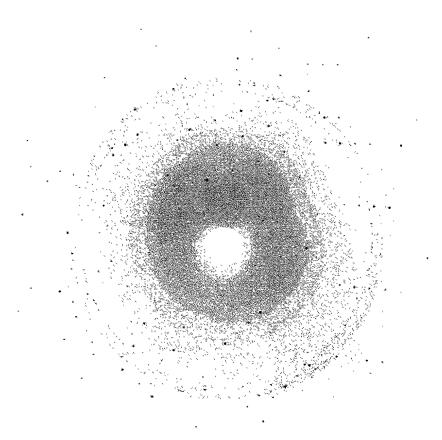
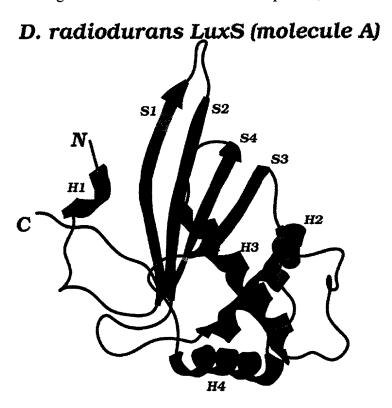
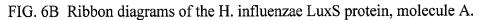
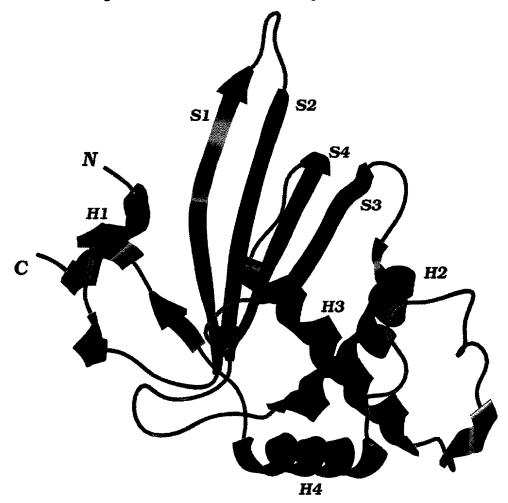


FIG. 6A Ribbon diagrams of the D. radiodurans LuxS protein, molecule A.

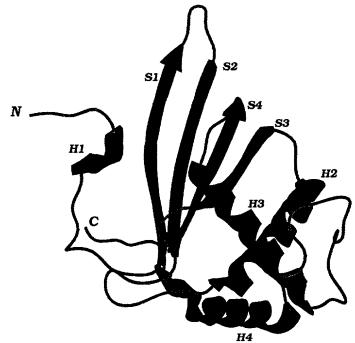






H. influenzae LuxS (molecule A)

FIG. 6C Ribbon diagrams of the H. pylori LuxS protein, moleculeB.



H. pylori LuxS (molecule B)

FIG. 7A Ribbon diagram of H. pylori LuxS as a dimer, the contents of the asymmetric unit.

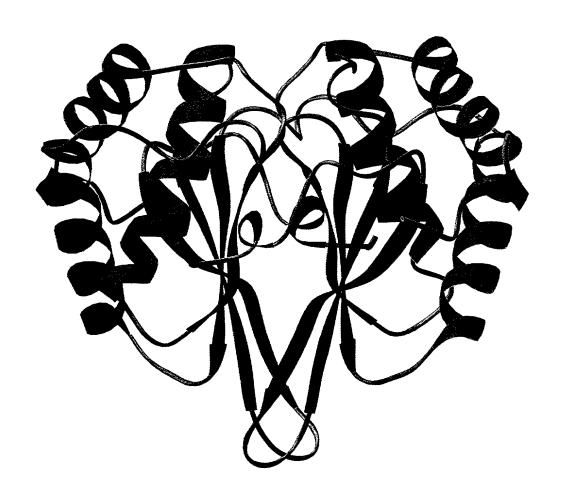


FIG. 7B Ribbon diagram of H. influenzae LuxS as a dimer with the bound methionines indicated in ball and stick.

## H. influenzae Dimerization

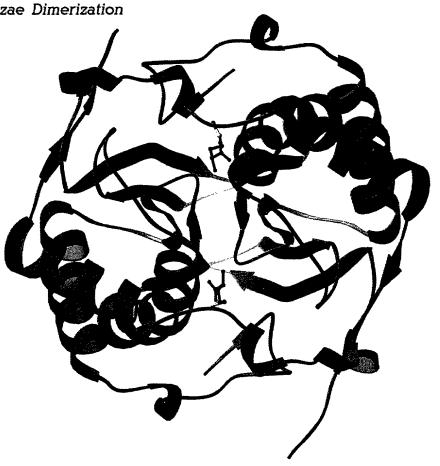


FIG. 8. Stereo image of C-alpha backbone of the H. pylori LuxS protein (same orientation as in FIG. 2A)

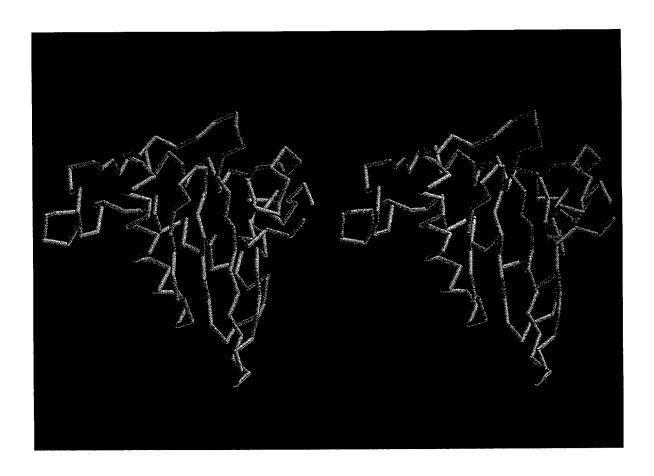


FIG. 9A Region of high sequence variability in LuxS as represented by Helix 3 (see FIG. 1). Helix 3 is the central (diagonal) helix closest to the observer.

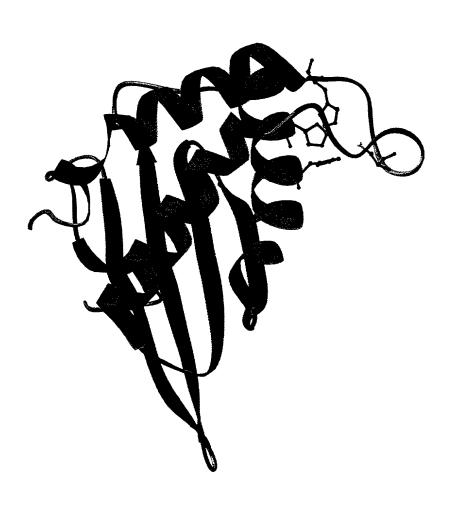


FIG. 9B Region of high sequence conservation in LuxS as represented by Helix 2 (see FIG. 1). Helix 2 is the central (vertical) helix closest to the observer.

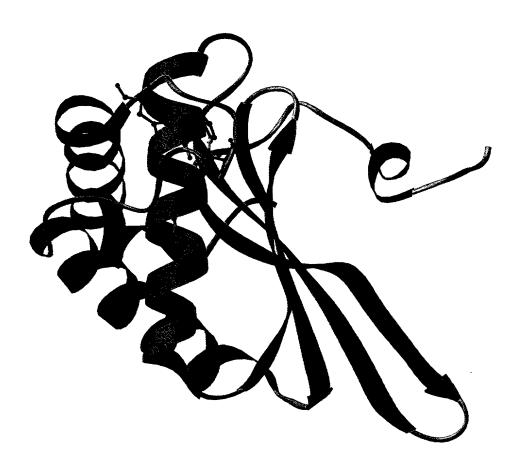


FIG. 10A The putative active site of LuxS.

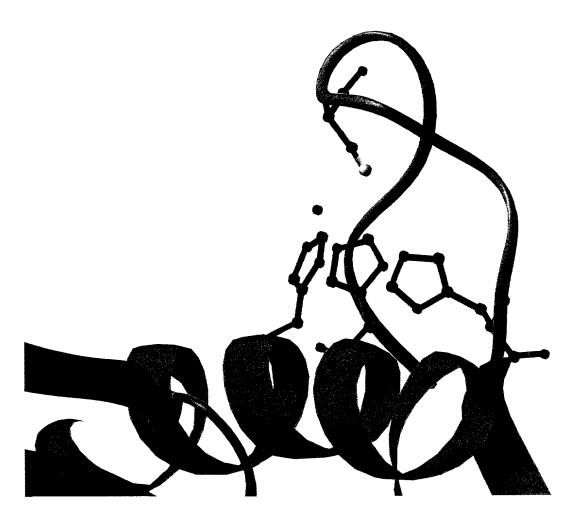


FIG. 10B The active site may enlarge through dimerization of LuxS molecules in vivo, as illustrated by the dimer found in the asymmetric unit of the LuxS crystal.



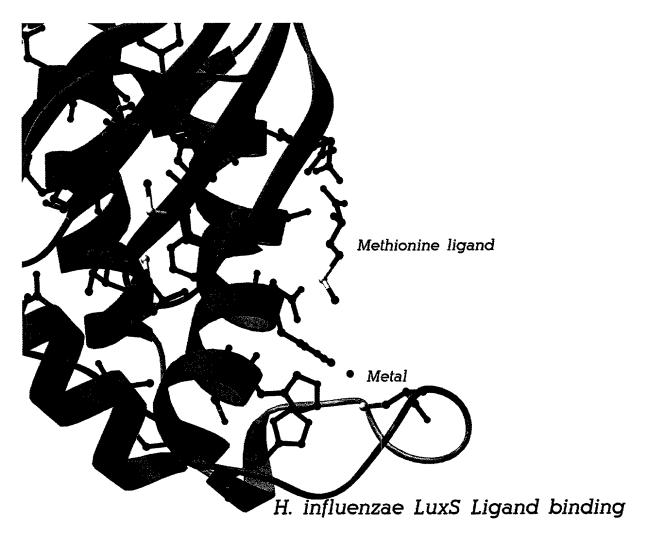


FIG. 11. Proximity of methionine binding site to metal binding site.

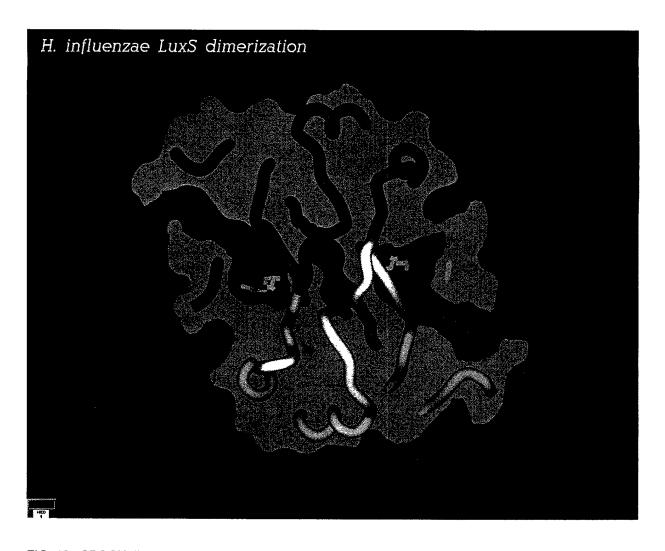


FIG. 12. SPOCK diagram of the molecular surfaces of the two molecule in the asymmetric unit of H. influenzae LuxS, cut away partially to reveal binding of the methionine ligands (ball and stick) and a channel through the opposing monomer leading out to the surface. A second channel to the binding site can also be seen. Worms represent the backbone atoms of the proteins in the cut away region.

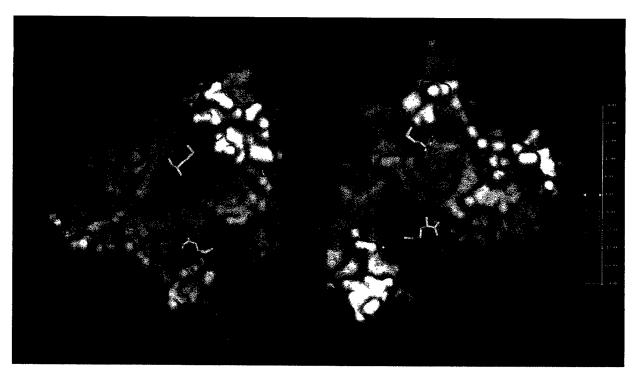


FIG.13A Molecular surface diagram of the H. influenzae LuxS dimer, separated and rotated to the viewer. The methionine ligand are represented as ball and stick, one per monomer with the virtual gold ligands representing where the methionine would lay across the opposing molecule. Red represent negative potential and blue positive potential. The charge complementarity of the dimerization is clear.

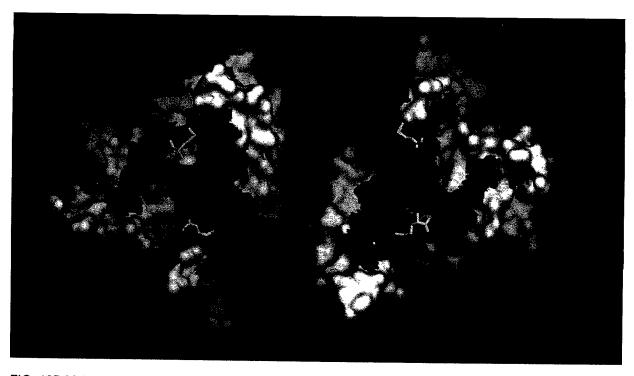


FIG. 13B Molecular surface diagram of the H. influenzae LuxS dimer, separated and rotated to the viewer. The methionine ligand are represented as ball and stick, one per monomer with the virtual gold ligands representing where the methionine would lay across the opposing molecule. Green represents conserved hydrophobic residues and red other conserved residues in the LuxS family (same as the color coding in FIG. 1).